2424-Pos Board B116
Dimerization of FGFR3 in Living Cells
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Receptor Tyrosine Kinases (RTKs) transduce biochemical signals via lateral dimerization in the membrane plane. Yet, our knowledge about the thermodynamics of RTK dimerization in cellular membranes is very limited. We are working to develop a FRET-based methodology to measure the dimerization free energies for RTKs in cells. We measure FRET with high spectral resolution, and we calculate the FRET efficiencies with high precision for each pixel of the cellular membrane. Here we present data for Fibroblast Growth Factor Receptor 3, an RTK that is critical for skeletal development. Since this receptor is activated by the ligand fgf1, we compare the FRET efficiencies with and without ligand. Consistent with the expectation that fgf1 stabilizes the FGFR3 dimer, we see an increase in the FRET signal.

2425-Pos Board B117
Probing the Interactions Between U24 from HHV-6A/7 and Fyn-SH3 or WW Domain Proteins
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U24 is a type II tail-anchored putative membrane protein unique to the *Roseolovirus* family, including HHV-6 and HHV-7. It contains an N-terminal proline-rich region and is believed to function by disrupting the signalling pathway in order to ensure the virus' survival. HHV-6A is a neurovirulent virus as it is often found in multiple sclerosis (MS) patients. It has also been shown to directly induce demyelination, a typical MS symptom, in naïve adult marmosets[1]. U24 from HHV-6A shares a seven residue identity with myelin basic protein (MBP), a protein responsible for the compaction of the myelin sheath in the CNS[2]. U24 from HHV-7, on the other hand, does not share this sequence identity with MBP, but this virus has also been implicated in MS, though indirectly[3].
In order to elucidate the exact role of U24 in MS, we have investigated the interaction of this protein with other partners such as the SH3 domain from Fyn tyrosine kinase and WW domain proteins. GST pull-downs, NMR titration data and molecular dynamics simulations will be presented. The differences in binding observed for U24 from HHV-6A and -7 will shed light into the hypothesis that U24 may function by mimicking MBP, as it has been previously shown that U24 and MBP can be phosphorylated in a similar manner[4].
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2426-Pos Board B118
Peptide NMR Studies of Cx26 Interdomain Interactions and their Regulation by Taurine
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Protonated aminosulfonates (AS), notably taurine, directly and reversibly inhibit homomeric and heteromeric channels that contain Cx26, a widely distributed connexin, but not homomeric Cx32 channels. Our previous biophysical studies identify the carboxyl-terminal domain of Cx26 (Cx26CT) as a key component of the channel modulation, suggesting a mechanism by which AS disrupts a pH-dependent association between the CT and cytoplasmic loop of Cx26 (Cx26CL), leading to occlusion of the pore [1,2]. Using recombinantly expressed Cx26CL and a Cx26CT peptide, NMR showed that taurine binds to the CL and not the CT, and that the CT and CL directly interact [3]. Our next goal to further characterize this mechanism is to identify the Cx26CL residues that directly interact with the Cx26CT and taurine. The Cx26CL construct used in the prior study (residues 98-139) contained a number of hydrophobic residues on the C-terminus, likely membrane associated, which decreased solubility below levels needed for detailed structural studies. Therefore, we created a shorter Cx26CL construct (residues 98-134) and were able to achieve millimolar soluble concentrations. Using this construct, we demonstrated by NMR a substantial interaction between the Cx26CT and the Cx26CL, which was blocked by taurine. Ongoing studies will determine (i) the binding affinities between the molecular partners, (ii) identify the Cx26CL residues involved in these regulatory interactions, and (iii) explore the potential effects of the Cx32CL and CT domains on these interactions, as may occur in Cx26-Cx32 heteromeric channels. Support: GM072631 & GM101950.
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2427-Pos Board B119
Design and Synthesis of a Novel Photochromic HDAC Inhibitor and its Photo Reversible Inhibitory Effect
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Core histone is composed of two H2A-H2B dimers and H3-H4 tetramers. Each histone have tail domain, which regulates dynamic structure of chromatin and transcription through posttranslational modifications. Histone acetylation-deacetylation is one of such epigenetic regulations of gene expression in eukaryotes. Histone deacetylase (HDAC) catalyze deacetylation of lysine residues in N-terminal domain of core histones and regulates gene transcription and expression. Inhibition of HDAC induces transcriptionally active chromatin, causing arrest of growth, differentiation and apoptosis. HDAC inhibitors are thought to be effective for neurodegenerative disorders, cardiac hypertrophy, inflammation and cancer. Using inhibitor composed of photochromic molecules, can be changed its property reversibly according to the wavelength of irradiated light, reversible site-directed regulation is thought to be achieved without displacement of solution or chemical modification to HDAC itself. In this study, we designed and synthesized three kinds of novel photochromic HDAC inhibitor, N-(2-hydroxy-5-phenylphenyl)-4-[(E)-2-phenyldiazen-1-yl]benzamide, N-phenyl-4-[(E)-2-phenyldiazen-1-yl]benzamide, and N-[4-({4-[(E)-2-phenyldiazen-1-yl]phenyl}formamido) butyl]-2-sulfanylacetamide composed of azobenzene moiety which may bind specifically to surface region, and benzamide or thiol group, which may bind to metal ion on active center , respectively. The design of the photochromic inhibitor was based on known HDAC inhibitor, which is known to affect HDAC class 1 selectively. Azobenzene moiety is placed in hydrophobic interacting region of inhibitor, therefore, it is expected that the light-induced structural change affects its affinity for HDACs. The synthesized photochromic HDAC inhibitor showed light-induced isomerization, derived from azobenzene moiety. Both of the cis and trans isomers of the inhibitor showed inhibitory effect of human whole HDAC activity. The trans-isomer of photochromic HDAC inhibitors showed higher inhibitory effect to HDAC in hela nuclear extract than cis isomer.